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TITLE: Randomized Trial of Interleukin-2 (IL-2) as Early Consolidation Following Marrow Ablative Therapy with Stem Cell Rescue for Matastatic Breast Cancer

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14. ABSTRACT
Interleukin-2 (IL-2) has the capacity to activate lymphocytes to kill multidrug resistant cancer cells. Our phase I data established the feasibility of administering a single course of low-dose IL-2 (1.6 million IU/m2/day as a continuous i.v. infusion for 18 days) as consolidation treatment to patients with metastatic breast cancer early after intensive chemotherapy. We are performing a phase II trial of AC+T chemotherapy followed by IL-2 consolidation (1 cycle as described above) in high-risk stage II and III breast cancer patients. Disease free survival and toxicity assessment represent major clinical aims (Specific aim 1). Immunologic effector mechanisms induced following MAT/SR by IL-2 infusion are being evaluated (Aim 2). This study opened 6/11/03. Fourteen patients have been accrued, all have completed planned treatment. Toxicity has been minimal. Laboratory correlation studies have been completed and the results of this trial will be submitted for publication in the next several months.

15. SUBJECT TERMS
Breast cancer, IL-2, immunotherapy, cytotoxicity, drug resistance

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Table of Contents

Introduction:

At least 46,000 women die from metastatic breast cancer each year in the United States[1,2]. Median survival remains 12-18 months from the diagnosis of metastatic disease, and progression-free survival beyond 5 years is rare (<10%)[3]. This has led to the testing of intensive chemotherapy, including marrow ablative therapy and stem cell rescue (MAT/SCR). Although this approach produced a high frequency of objective responses in patients with metastatic breast cancer, with up to 40-50% complete responses, responses tend to be short-lived. Only a minority of women (10-20%) achieved long-term disease free survival. Relapses may have be due to both minimal tumor contamination of stem cells reinfused into patients, as well as residual chemotherapy resistant tumor cells not cleared by the MAT/SCR regimen. IL-2 activated lymphocytes, termed lymphokine-activated killer (LAK) cells have significant cytotoxic activity against autologous breast cancer cells and breast cancer cell lines. Our own studies have demonstrated that multidrug-resistant tumor cells remain sensitive to LAK cell mediated killing.

We performed a phase I trial to test the feasibility of administering a single course of low-dose IL-2 (1.6 million IU/m^2/day as a continuous i.v. infusion) as consolidation treatment to patients with metastatic breast cancer early after MAT/SCR. This study established that IL-2 consolidation could be safely begun starting on day +14 post MAT/SCR with minimal toxicity. Substantial LAK cell induction was observed, using flow cytometric and cytotoxicity assays. Thus far, only 3 of 10 patients have had breast cancer relapse or progression, and a small second breast cancer was detected in 1 patient. Seven patients (60%) remain in complete remission at a median of 435 days (range: 224 - 720 days) post stem cell transplantation.

Patients with locoregional breast cancer with adverse characteristics, such as tumor size > 5cm with 1-3 positive lymph nodes, any tumor size and ≥4 lymph nodes involved, tumor invading through lymph node capsule, involvement of internal mammary lymph nodes, chest wall or skin infiltration and inflammatory characteristics also have a poor prognosis. For example, women with 4-10 lymph nodes involved have an approximately 40% 10 year disease-free survival (DFS), while women with >10 lymph nodes positive have an approximately 20% 10 year DFS[1,4]. Patients with Stage IIIA and IIIB breast cancer have 5 year survivals of 40-50%[1,5]. Survival with inflammatory breast cancer with aggressive multi-modality therapy is estimated to be 50% at 5 years and 35% at 10 years[1]. Methods to improve these results, incorporating novel therapeutics, are of significant importance for the healthcare of these patients.

In this application we proposed to perform cytoreduction in patients with metastatic breast cancer using doxorubicin/cyclophosphamide plus taxane chemotherapy, followed by an 18 day continuous infusion of low-dose IL-2 starting on day +14 to activate lymphocytes to kill residual chemotherapy-resistant cancer cells (the grant objectives were revised in 2003). Based on preliminary data, we hypothesize that a single course of IL-2 will result in a significant improvement in disease-free survival, with minimal toxicity. Effectiveness of this approach may correlate with the effective induction of LAK precursor and effector cells, as well as evidence for reduction in the burden of minimal residual cancer cells. In **Specific Aim 1** we will perform a prospective Phase II clinical trial to test whether the addition of 1 cycle of continuous i.v. infusion of IL-2 in women with metastatic breast cancer, starting on day +14 after treatment with AC+T

chemotherapy, to assess toxicity and to estimate progression-free and overall survival. In **Specific Aim 2** we will evaluate possible immunologic effector mechanisms induced following MAT/SCR and IL-2 infusion. Phenotypic and functional assays for LAK cell induction and enzyme immunoassays for circulating pro-inflammatory cytokines will be performed.

Body:

Initiation of our initial clinical trial proposed was delayed by a number of unanticipated events. First, shortly after this proposal was funded in 1999, a series of randomized trials was reported at the American Society of Clinical Oncology meetings in 5/00 comparing standard dose chemotherapy and marrow ablative therapy and stem cell rescue (MAT/SCR) for treatment of advanced breast cancer. The conclusions of all but one of these trials was that there was no advantage to stem cell transplants in breast cancer patients over standard chemotherapy[6-8](1-3). The second event was that the one trial showing benefit of MAT/SCR over chemotherapy (Bezwoda, et al) was found to contain fraudulent data[9]. In combination, these findings made our clinical trial including MAT/SCR unacceptable. Since the goal of MAT/SCR in our trial was to provide maximal cytoreduction prior to IL-2 based immunotherapy, this goal was still felt scientifically reasonable, given our impressive phase I trial results. In order to further prove the validity of these observations, we felt that a change from a randomized trial to a single arm phase II study (MAT/SCR followed by an 18 day infusion of IL-2) was warranted. This change was discussed with the USAMRMC and the study protocol and consent documents were rewritten. A third point holding up the clinical trials was due to negotiations between the University of Utah lawyers and the USAMRMC concerning required liability clauses in the consent document and final approval by the University of Utah IRB. After many months of negotiations, a finalized consent language and protocol was agreed upon. A final draft has been submitted to the University of Utah IRB and was approved with minor revisions. Further investigation into fraudulent data published by Bezwoda was presented at the ASCO meetings in 5/01. This resulted in the general abandonment of MAT/SCR as a breast cancer treatment modality in the United States. At the end of August of 2001, I was notified by my co-investigator Dr. Peterson that we would not be able to accrue any patients to a MAT/SCR regimen based trial, since patient referral to the University of Utah BMT program had virtually stopped.

We therefore re-evaluated our options. Due to our exciting preliminary results, we still strongly believe that the concept of IL-2 consolidation in high-risk breast cancer should be tested after maximal cytoreduction. Given the apparent equivalence of MAT/SCR and standard chemotherapy in high risk breast cancer patients, we concluded that an alternative method to test our hypothesis is to enroll high-risk breast cancer patients who are treated systematically with surgery, followed by a standard chemotherapy regimen (doxorubicin/cyclophosphamide followed by paclitaxel or docetaxel)[10,11], followed by a 18 day infusion of IL-2. Patients will subsequently receive irradiation to the breast and regional node areas. Patients deemed at high risk include: patients with ≥4 lymph nodes positive (40% 5 year survival with >4+ nodes, <20% 5 year survival with >10 nodes involved), inflammatory breast cancer (<20% 5 year survival) and patients with resected stage IV disease (Stage IV NED, <10-20% 5 year survival)[12].

Two breast cancer medical oncology specialists (Dr. John H. Ward and Dr. Saundra

Buys), from the Huntsman Cancer Institute were added as co-investigators to increase accrual (to replace Dr. Petersen, a MAT/SCR specialist). We also obtained a commitment from Chiron to provide recombinant IL-2 for this trial. The protocol finally opened to patient accrual 6/11/03 at the Huntsman Cancer Institute. Fourteen patients have been enrolled, and 14 have completed planned therapy. The patient characteristics are shown (**Table 1**). All had regionally advanced breast cancer with a high risk of relapse. All patients were treated with adjuvant chemotherapy consisting of cyclophosphamide and doxorubicin (AC) followed by paclitaxel (T, Taxol). Most were treated in a dose-intense fashion, using hematopoietic growth factor support. Median progression-free survival is 596 days (range 136-1337), and median overall survival is 874 days (range 136-1337 days) from onset of AC+T chemotherapy. With a median follow-up of 739 days, only two patients in this high-risk population have experienced a breast cancer relapse (this appears lower than expected). One patient developed a non-Hodgkin's lymphoma as a second malignancy. Relationship to treatment is uncertain. There has been 1 death (due to breast cancer). Toxicity to date has been minimal (no Grade III or IV toxicity was noted)(**Table 2**). One patient had a brief interruption in treatment due to fever, which subsided upon discontinuation of IL-2 and the patient was able to complete planned treatment. Clinical samples were submitted to the laboratory for testing on all 11 patients. Our results indicate marked induction of circulating LAK cells in all but 3 tested patients, comparing pretreatment and immediately post-treatment with IL-2 (**Figures 1A and B**). In addition, a variable pool of IL-2 responsive NK precursor cells (**Figures 2A and B**) was seen pretreatment. Cytotoxicity derived from this population of cells was markedly increased in all patients following IL-2 infusion. There was expected variability in maximum cytotoxicity between patients, comparing specific lysis at a 100:1 effector to target cell ratio. It will be interesting to see if patients with greatest pretreatment cytotoxicity or IL-2 induced increase in cytotoxicity exhibit long-term progression-free survival upon longer follow-up.

Due to low accrual of this relatively infrequent breast cancer patient population, this study has now been closed. These preliminary results will be submitted for publication. Hopefully, our phase II results will encourage a larger scale study to validate these findings. The Huntsman Cancer Institute Clinical Trials Office has committed to provide the resources for the long-term follow-up patients beyond the scope of the current grant funding.

Key research accomplishments:

We treated 20 patients with MAT/SCR in our phase I pilot trial. Patients received IL-2 either starting on day 1 (10 patients) or day 14 (10 patients), following stem cell infusion. A total of 17 patients were evaluable for response at the time of initial analysis. A total of 17 patients (85%) completed the IL-2 course. Three patients receiving IL-2 from day 1 required IL-2 infusions to be terminated early (2 fever, 1 thrombocytopenia). Relapse free survival was 45% with 580 day median follow-up (135-1175 days), with 75% overall survival.

LAK cell activation was evaluated in patients undergoing IL-2 infusions starting either day 1 (5 patients) or day 14 post stem cell infusion (5 patients). Cytotoxicity against the MCF-7 breast cancer line was detected in all patients, regardless of whether the IL-2 infusion was started day 1 or 14. Increased cytolytic activity was detected in cytotoxicity

assays performed with the addition of IL-2, suggesting a substantial increase in circulating LAK cell precursors in both patient populations. Phenotypic evaluation established that while CD56+ cell populations were expanded in both patient groups, the absolute number of circulating CD56+ cells was 10-fold higher in patients receiving IL-2 starting on day 14.

In the current phase II study, 14 patients with high-risk regionally advanced breast cancer were treated with AC+T chemotherapy, followed by an 18-day low dose continuous infusion of IL-2. This appeared to result in a low relapse rate (2/14 patients) with almost 2-year follow up. Treatment related toxicity has been minimal (none > grade 2). All patients demonstrated activation of lymphokine activated killer cells following completion of the IL-2 infusion. Low dose IL-2 infusion is feasible and safe after AC+T chemotherapy and is showing a promising effect on breast cancer relapses. This regimen should be further evaluated in high-risk breast cancer patients.

Reportable outcomes:

Abstract presented at Era of Hope Meeting 9/25/02-9/28/02 (enclosed)

Petersen FB, **Samlowski WE**. Feasibility of low dose continuous infusion of IL-2as a consolidation treatment following intensive breast cancer chemotherapy. Proceedings Era of Hope DOD Breast Cancer Research Program Mtg. 2002, p 33-20

Conclusions:

The proposed use of IL-2 following maximal cytoreduction of tumor by standard chemotherapy or MAT/SCR is quite promising based on our preliminary data, with 45% of patients achieving >2 year disease free survival after MAT/SCR plus IL-2 and 12 of 14 patients disease free of breast cancer recurrence on the current trial with a median of almost 2 years of follow-up. Longer-term follow-up will be needed to more critically assess the benefit of IL-2 infusion following breast cancer chemotherapy. Preliminary data shows IL-2 related toxicity to be minimal and feasibility of immunologic monitoring. A manuscipt describing these findings is currently being prepared for submission.

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Appendix

Table 1: Patient Characteristics

Patient Initials	Age at Diagnosis	TNM Stage	Start AC	End AC	Start T	End T	Start IL-2	End IL-2	Status	Last F/U date	PFS (from ACT)	OS (from ACT)	Grade 1 Toxicities	Grade 2 Toxicities
BAW	38	T4 pN2a M0	1/31/03	4/4/03	4/25/03	6/27/03	7/11/03	7/29/03	NED (NHL)	9/29/06	1337	1337	Diarrhea, hypoglycemia, hypocalcemia, stomatitis, skin irritation (port), nasal congestion, chest pain	fatigue, dyspnea
S-W	40	T2 N1biv M0	4/2/03	6/6/03	6/27/03	8/29/03	9/12/03	9/30/03	5/18/04	4/18/05	412	747	fatigue, nausea, myalgia	none
C-N	51	T2 N1bii M0	5/6/03	7/8/03	7/29/03	9/30/03	10/14/03	11/1/03	NED	8/22/06	1204	1204	myalgia, SGPT	hyperglycemia
L-Z	66	T1 N3 M0	11/21/03	1/2/04	1/16/04	2/27/04	3/12/04	3/30/04	NED	6/16/06	938	938	edema, fatigue	none
L-A	47	T2 N3 M0	1/2/04	2/13/04	2/27/04	4/9/04	4/23/04	5/11/04	NED	6/30/06	910	910	hemoglobin, neuropathy	extravasation (port site), chest pain
EPW	29	T2 N3 M0	1/15/04	2/26/04	3/12/04	4/22/04	5/16/04	6/2/04	7/18/05	10/27/06	550	1016	hemoglobin, skin (flushing)	none
J-B	51	T2 N3 M0	4/7/04	5/21/04	6/4/04	7/30/04	8/20/04	9/7/04	NED	8/29/06	874	874	itching, nausea, chills	edema, fatigue, myalgias
S-S	54	T1cN3aM0	2/12/04	3/25/04	4/8/04	5/20/04	6/11/04	6/29/04	NED	10/17/06	978	978	hemoglobin, myalgia, fatigue	eosinophilia
S-E		T3 N2 M0	5/10/04	6/22/04	7/6/04	8/18/04	9/8/04	9/26/04	NED	12/27/05	596	596	hemoglobin, fatigue, myalgia, SGOT	neuropathy (ongoing from Taxol)
R-B	42	T3N3aMo	9/30/04	11/1/04	11/24/04	1/6/05	1/29/05	2/16/05	NED	5/16/06	593	593	hemoglobin, diarrhea, fatigue, platelets, nausea	WBC
C-C	39	T2pN1biiM0	5/5/05	6/17/05	7/1/05	8/12/05	9/2/05	9/20/05	NED	7/5/06	426	426	psoriasis, hyperglycemia, hypotension	Thrombosis (PICC related), eosinophilia
L-A	64	T4a Nx Mx	4/1/05	5/20/05	7/12/05	9/2/05	11/1/05	11/19/05	NED	6/23/06	448	448	fever, myalgia	eosinophilia
J-J	49	T2 N2a M0	4/11/06	5/23/06	6/6/06	7/18/06	8/2/06	8/20/06	NED	8/25/06	136	136	fatigue, nausea, myalgia	none

Table 2: Toxicity related to IL-2 infusion

Toxicity Summary Report

Study Name: "AC +T Chemotherapy Followed by an 18 day Low Dose Infusion of IL-2 as Adjuvant Treatment for Women with HRBC

PI: Wolfram Samlowski, M.D.
Coordinator: Gina Gregovich

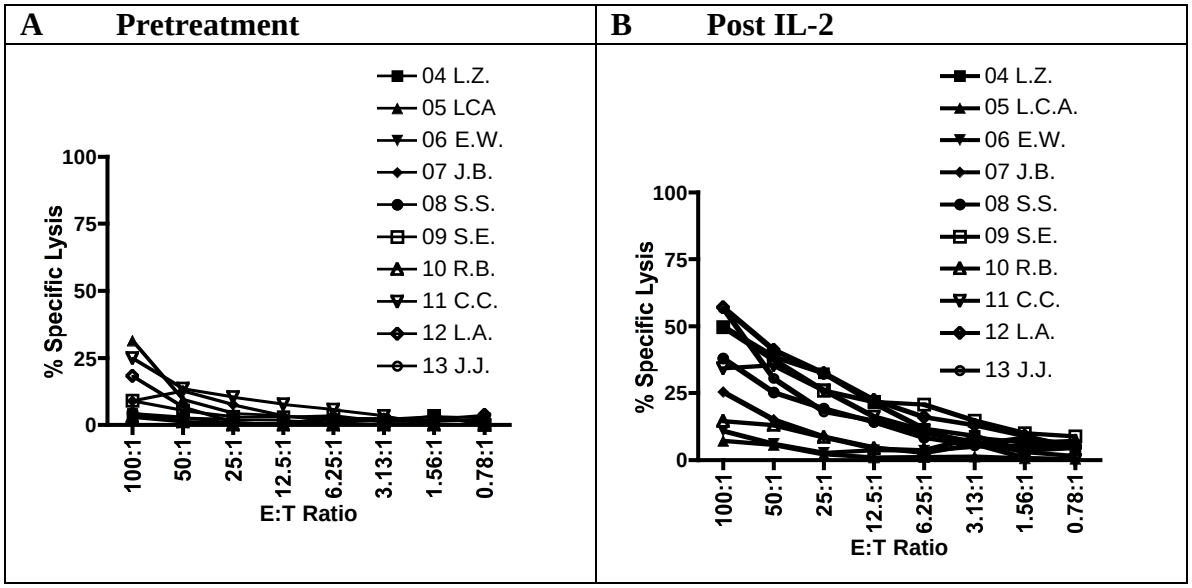
Total Enrolled: 14
Number of subjects for which toxicities are being reported: 14

	For the Month of: October 2006									**Entire Reporting Period:Dec 2003 - October 2006**				
				Reported to IRB?(y/n)		Reported to IRB?(y/n)								
TOXICITY	**CTC Grade**									**CTC Grade**				
	1	**2**	**3**		**4**		**5**			**1**	**2**	**3**	**4**	**5**
Allergy/Immunology														
Auditory/Hearing														
Blood/bone marrow (thrombocytopenia)														
Blood/bone marrow (eosinophilia)											**2**			
Blood/bone marrow (hemoglobin)														
Blood/bone marrow (ANC/leukocytes)														
Cardiovascular (edema)										**1**	**1**			
Cardiovascular (tachycardia)										**1**				
Cardiovascular (hypotension)										**1**				
Coagulation (PTT)														
Constitutional symptoms (chills)										**1**				
Constitutional symptoms (fatigue)	**1**									**6**	**2**			
Constitutional symptoms (fever)										**2**				
Death														
Dermatology other: psoriasis										**1**				
Dermatology/Skin (itching)										**1**				
Dermatology/Skin (irritation at port site)										**1**				
Dermatology/Skin (extravasation)											**1**			
Dermatology/Skin (flushing)										**1**				
Endocrine														
Gastrointestinal (diarrhea)										**2**				
Gastrointestinal (nausea/vomiting)	**1**									**4**				

Hemorrhage														
Hepatic (transaminitis)														
Infection/Febrile Neutropenia														
Lymphatics														
Metabolic/laboratory (acidosis)														
Metabolic/laboratory (bicarbonate)														
Metabolic/laboratory (hypocalcemia)										**1**				
Metabolic/laboratory (hypoglycemia)										**1**				
Metabolic/laboratory (hyponatremia)														
Metabolic/laboratory (hypoalbuminemia)														
Metabolic/laboratory (hypokalemia)														
Metabolic/laboratory (hyperglycemia)										**1**	**1**			
Metabolic/laboratory (hyperkalemia)														
Metabolic/laboratory (hypermagnesemia														
Metabolic/laboratory (transaminitis)										**1**				
Musculoskeletal (aches)		**1**								**4**	**2**			
Neurology														
Ocular/Visual														
Pain														
Pulmonary (ARDS)														
Rena/Genitourinary (creatinine)														
Secondary Malignancy														
Sexual/Reproductive Function														
Syndromes														
Vascular (Thrombosis-vascular access related)											1			

***Instructions**: Document toxicity at its highest grade. For instance if toxicity goes from a grade 2 to a grade 3, erase it from the grade 2 column and put it in the grade 3 column. If a toxicity is intermittent, it should be reflected on the table only once. List the toxicity as follows: General CTC Category with specific AE in parenthesis.*

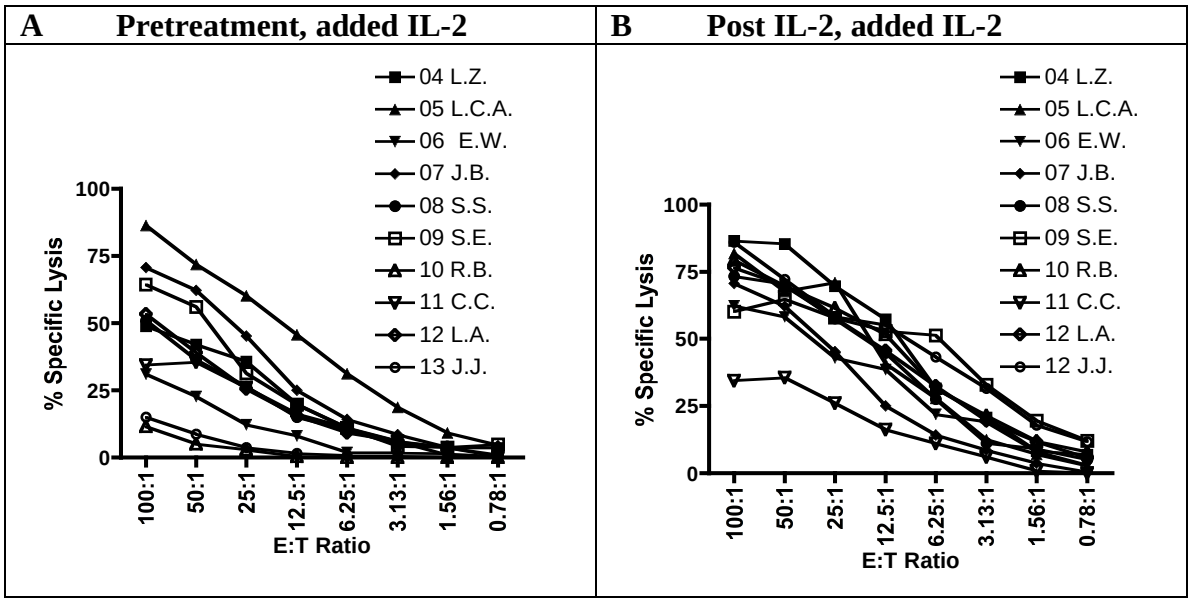
Figure 1: Pretreatment and post treatment LAK cell cytotoxicity



Assay for tumor cell killing by cells. Lymphocyte mediated cytotoxicity was assessed using a 4 hour chromium release assay as previously described (Samlowski WE, Petersen R, Cuzzocrea S, et al. A nonpeptidyl mimic of superoxide dismutase, M40403, inhibits dose- limiting hypotension associated with interleukin-2 and increases its antitumor effects. *Nat Med.* 9:750-755, 2003.) Briefly, MCF-7 cells (a NK-resistant breast cancer cell line) were incubated with 100 μCi ^{51}Cr per 10^8 cells for 60 min. Following extensive washes in complete RPMI medium, a constant number of labeled target cells (10^4 cells/well) were added to serial dilutions of lymphocytes (obtained by Ficoll-Hypaque density separation (starting at a 100:1 effector to target cell ratio) in a microtiter plate, for 4h. Specific cytotoxicity was calculated from the average of triplicate wells by the formula:

$$\text{Specific cytotoxicity} = \frac{\text{experimental cpm - spontaneous release cpm x 100}}{\text{total release cpm - spontaneous release cpm}}$$

Figure 2: Detection of LAK precursor activity following IL-2 addition



Parallel wells, with addition of 6000 IU/ml IL-2 were set up, to test for rapid up-regulation of NK to LAK activity which is found when IL-2 receptor-expressing cytolytic cells are present in peripheral blood leukocyte population.